Inhibition of the Microbial Growth in Hake, Megrim and Angler by Storage in an Icing System with Preservative Organic Acids

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Abstract. *Natural preservative organic acids (ascorbic, citric and lactic acids) were used to prepare a novel organic acid-flake icing system and evaluated for the preservation of three comercially-relevant marine species during refrigerated storage: hake, Merluccius merluccius, megrim, Lepidorhombus whiffiagonis and angler, Lophius piscatorius. The icing system was prepared with fresh water including two different concentrations of a commercial acid mixture-formula containing the three organic acids at 800 ppm and 400 ppm (C-800 and C-400 batches, respectively). Aerobic mesophiles, psychrotrophes, proteolytic bacteria, trimethylamine and pH value were evaluated in fish muscle throughout storage, the results being compared with sensory analysis. Significantly (P<0.05) lower counts of mesophiles were determined for hake and megrim in both C-800 and C-400 batches, as compared with the C-0 control batch. In the case of angler, significantly (P<0.05) lower counts of mesophiles, psycrotrophes and proteolytic microorganisms (P<0.05) were determined as a result of employing the C-800 icing conditions. Both C-400 and C-800 megrim batches exhibited significantly (P<0.05) lower pH values than the C-0 control batch, a result that was also observed in the C-800 angler batch. These results were in agreement with sensory analysis, that revealed a shelf life extension of the three fish species in the C-800 batch. According to the parameters assessed, storage of hake, megrim and angler in the proposed ice system containing natural organic acid preservatives allows a better maintenance of their microbial and sensory quality.*

Keywords. fish quality, fish spoilage, fish storage, refrigeration, organic acids, icing sytems, microbial quality, sensory quality.

Introduction

Marine species represent perishable products whose quality and freshness rapidly decline post-mortem due to a variety of microbial and biochemical degradation mechanisms (Pigott & Tucker, 1990; Whittle, Hardy, & Hoobs, 1990). Thus, significant deterioration of sensory quality and nutritional value undergoes in chilled fish as a result of different damage pathways, such as endogenous enzymatic activity, microbial development and lipid oxidation mechanisms (Olafsdóttir et al., 1997; Whittle, Hardy, & Hoobs, 1990). With the aim of slowing down the mechanisms involved in quality loss, the fish specimens should be refrigerated immediately after capture. Therefore, fish has traditionally been cooled and stored in either flake ice (Nunes, Batista, & Morâo de Campos, 1992), refrigerated sea water (Kraus, 1992), ice slurries (Barros-Velázquez, Gallardo, Calo, & Aubourg, 2008; Rodríguez, Barros- Velázquez, Piñeiro, Gallardo, & Aubourg, 2006; Rodríguez, Losada, Aubourg, & Barros-Velázquez, 2004) or preserved by exposure to chemical agents (Hwang & Regenstein, 1995). Among chemical treatments, natural organic acids have shown to represent a relevant choice because of their easy availability, low commercial cost as well as by providing antioxidants and antimicrobial properties in fish stored (Aubourg, Pérez-Alonso, & Gallardo, 2004; Alakomi et al., 2000; García-Soto, Sanxuás, Barros-Velázquez, Fuertes-Gamundi, & Aubourg, 2010).

The present work was aimed at studying the effects of an organic acid-icing system (including ascorbic, citric and lactic acids in icing medium; AA, CA and LA, respectively) on the microbial development and shelf-life of three commercially-relevant fish species (hake, *Merluccius merluccius*; megrim, *Lepidorhombus whiffiagonis*; and angler, *Lophius piscatorius*) from the Grand Sole North Atlantic fishing bank during refrigerated storage.

Materials and Methods

The organic acid-icing mixture was elaborated by preparing flake ice with water including two different concentration of a commercial formula (800ppm and 400ppm; C-800 and C-400 conditions respectively). The icing system was applied to hake, *Merluccius merluccius*; megrim, *Lepidorhombus whiffiagonis*; and angler, *Lophius piscatorius.* Comparison to fish specimens kept under traditional ice prepared only from water (control) was performed in parallel. Fish specimens were surrounded by the corresponding ice at 1:1 fish-to-ice ratio and placed in a refrigerated room (4ºC). Boxes employed allowed draining and ice was renewed when required. Fish samples from the different icing conditions were taken for analysis on days 1, 5, 8, 12 as well as at the starting day; day 15 was also considered in the case of hake. The study was addressed to evaluate microbial development related to shelf life and quality. The following microbial groups were considered: total aerobes were investigated by surface inoculation in plate count agar (PCA, Oxoid Ltd., London, UK), after incubation at 30ºC for 48 h. Psychrotrophes were also investigated in PCA but incubation was carried out at 7–8ºC for 7 days. Microorganisms exhibiting a proteolytic phenotype were investigated in casein-agar medium after incubation at 30ºC for 48h. Trimethylamine-nitrogen (TMA-N) values were determined by the picrate method, as previously described by (Tozawa, Erokibara, & Amano, 1971). The evolution of pH value along storage time was determinated by means of a pH metter. A complementary sensory evalutation (eyes, gills, external odour, muscle odour and taste) was carried out according to EC guidelines (Council Regulation, 1990).

For all kinds of fish species and icing conditions, three different batches (n=3) were considered and analysed separately troughout the whole experiment. Data were subjected to statistical analysis ($p<0.05$) to explore significant differences as a result of packaging conditions and frozen storage time (SPSS inc., Chicago, II, USA).

Results

Microbiological analyses

The comparative evolution of aerobic mesophiles along storage time in case of hake and megrim evidenced significant ($P<0.05$) lower mesophiles counts in C-800 and C-400 systems, as compared with the C-0 control batch. On the other hand, angler stored in C-800 system, exhibited significant ($P<0.05$) lower mesophiles counts than C-400 and C-0 batches. With respect to psychrotrophes, statistically-significant ($P<0.05$) lower counts were determined on angler C-800 batch as compared with the C-400 and C-0 batches, and counts of proteolytic microorganisms in angler stored in C-800 system were significant ($P<0.05$) lower as compared with the other two batches.

TMA-N evolution

In the case of hake, the C-800 batch exhibited lower levels of formation of TMA-N throughout storage as compared with the other two batches, a result that indicates that the combination of organic acids at this proportion is benefitial due to the slowing down of the microbial production of these amines. In the case of megrim, the inclusion of organic acids in the icing system did not provide any significant advantage in terms of TMA-N formation. Finally, in the case of angler, the incorporation of CA, AA and LA in the C-800 batch provided significant protection as regards TMA-N formation as compared with the C-0 batch at all sampling times. Moreover, the C-400 batch including intermediate concentrations of these natural organic acids was also favourable as compared with the C-0 batch.

pH value analyses

pH values of megrim muscle stored in the C-800 and C-400 systems were significantly ($P<0.05$) lower as compared with the C-0 control batch. This indicating that the incorporation of natural preservatives to the icing system, at any of the two concentrations tested, implied a significant reduction of microbial and endogenous alkalinizing mechanisms that may limit fish shelf life. Likewise, in the case of angler, the pH value of muscle was significantly ($P<0.05$) lower as a consequence of storing such fish species in the C-800 icing system, as compared with the C-400 and C-0 control batches.

Sensory analyses

In the case of hake, the C-800 batch, incorporating the highest concentration of organic acids, showed the best behavior, retaining acceptable quality even after 12 days of storage, a result that was not observed in the other two batches. In the case of megrim, only the C-800 batch allowed acceptable quality after eight days of storage, as compared with the other two icing batches. Finally, angler specimens exhibited a better behavior in all batches as compared with hake and megrim. However, the C-800 batch maintained very good sensory quality up to day eight, while the other batches were considered to be acceptable at that time. Moreover, angler specimens stored in the C-800 organic acid-icing system were acceptable up to day 12, a result that was not observed for the other two batches.

Conclusions

The microbial and sensory results obtained in this study allow to conclude that such novel icing system implied the slowing down of microbial growth in hake, megrim and, especially in angler, these results being in agreement with a significant extension of the fish shelf life. These results

may be of practical interest for achieving a better preservation of fish quality not only in land but also in on-board strategies.

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